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(54) Determining the concentration of water soluble species in biological fluid.

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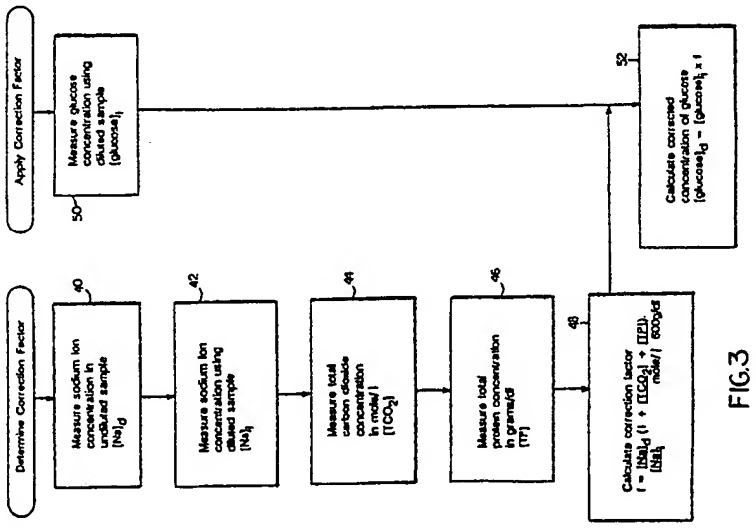

FIG.3

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DETERMINING THE CONCENTRATION OF WATER SOLUBLE SPECIES IN BIOLOGICAL FLUID

This invention relates to determining the concentration of water soluble species in a biological fluid.

Sodium and potassium ion concentrations in blood plasma, for example, can be measured directly (by direct potentiometry using an ion selective electrode) or indirectly (by flame photometry or indirect potentiometry procedures, which involve sample dilution). Methods which involve diluting the sample, however, tend to underestimate the concentration of water soluble species because the plasma water fraction of the sample (which includes the water soluble species and represents only a portion of the whole sample) is effectively diluted more than are the separate, purely aqueous, calibrating solutions used in the measurement. The effect increases with increasing protein or lipid concentration, e.g., in pathological samples (Shyr et al., Clin. Chem. 26 :1517 (1980); Coleman et al., Clin. Chem. 27 ; 1938-1939 (1981)).

Summary of the Invention

In general, the invention features correcting an initial measurement of the concentration of a first water soluble species dissolved in a water-based component of a biological fluid that also includes a second water soluble species and a volume occupying component, the initial measurement having been taken using an original sample of the fluid that had been diluted by an amount of additional aqueous solution to form a diluted sample; the concentration of the second water soluble species is measured in an undiluted sample of the biological fluid to obtain a direct concentration and measured using a diluted sample of the fluid to obtain an indirect concentration, and the initial measurement of the first species is adjusted based on a combination of the direct and indirect concentration measurements of the second species.

In preferred embodiments, adjusting the initial measurement of the first species includes generating a correction factor by forming a ratio of the direct and indirect measurements of the second species, the biological fluid is blood plasma or serum, the first water soluble species is glucose, and the second water soluble species is sodium ion. The blood plasma contains additional water soluble species (e.g., carbon dioxide and protein) which complex with the second water soluble species, and the correction factor is adjusted accordingly. Calculation of the correction factor (f) involves measuring a direct concentration of sodium ion in a sample of undiluted plasma, measuring a indirect concentration of sodium ion using a diluted sample, measuring the concentration of total carbon dioxide in the sample, measuring the concentration of total protein in the sample, and substituting the measured concentrations into the equation: $f=([Na]_d/[Na]_i) (1 + [TCO_2]/mole/l + [TP]/600g/dl)$.

This system permits rapid, accurate determination of the concentration of, e.g., glucose in blood plasma based on indirect measurements. The correction factor accounts for fluctuations in concentration of species that interfere with the calculation of an appropriate sample dilution ratio.

Other advantages and features will become apparent from the following description of the preferred embodiment.

We first briefly describe the drawings.

Fig. 1 is a schematic diagram of a prior proposed method of calculation of the plasma water dilution ratio.

Fig. 2 is a schematic diagram of analyzer apparatus.

Fig. 3 is a flow diagram of a method of calculating the corrected concentration of glucose.

To determine indirectly the concentration of glucose in blood, a small sample of blood plasma is diluted by a known amount of water or diluent, the concentration of the glucose in the diluted sample is measured, and the measurement is adjusted by the dilution factor (the ratio of the diluted sample volume to the volume in which glucose was originally dissolved) to give the concentration of glucose in the original sample. The concentration of a species determined in this manner is known as the indirect concentration.

Referring to Fig. 1, it can be seen that because blood plasma 10 includes a fraction 12 containing volume occupying (VO) species (e.g., lipids or the hydrophobic regions of proteins) in addition to the fraction 14 containing water soluble (WS) species (e.g., electrolytes such as sodium or potassium ions and non-electrolytes such as glucose, urea, or cholesterol), the simple ratio of diluted volume to original sample volume will not produce an accurate dilution factor for the plasma water fraction. For an aqueous standard 10a, the water soluble portion 14a is the entire sample volume of 100 µl. For a plasma sample, the fraction 12 containing VO species represents about 6% of the total volume, or 6 µl out of a 100 µl sample.

If 100 µl of aqueous standard is diluted 1:200, an aqueous standard dilution ratio 18 can be calculated

as

$$\frac{\text{final volume}}{\text{initial volume}}, \quad \frac{20{,}000\ \mu l}{100\ \mu l} = 200.$$

A plasma water dilution ratio 20 for a sample of blood plasma, calculated in the same manner, is equal to

$$\frac{19{,}900\ \mu l + 94\ \mu l}{94\ \mu l} = 212,$$

or a difference of 6%. This calculation of a plasma water dilution ratio is dependent upon the ability to measure the volume VO. This volume can vary from individual to individual and can be significantly larger in pathological blood plasma samples.

A method has been developed for determining a correction factor to adjust the concentration of a water soluble species in blood plasma that eliminates the need for measuring the actual sample volume of the volume occupying fraction. Instead, a correction factor is calculated based on the ratio of direct to indirect concentration determinations for a first water soluble species (e.g., sodium ion), and that factor is used to correct the indirect concentration determination of a different water soluble species (e.g., glucose). Adjustments can be made to the correction factor to account for the formation of interfering complexes.

Example

Referring to Fig. 2, a known volume 30 of blood plasma from a patient is placed into a sample cup and aspirated into analyzer 32. Another sample 34 of known volume of plasma from the same patient is diluted by a known amount with an aqueous solution in diluter 36, and diluted sample 38 is then also aspirated into analyzer 32.

If sodium ion is the water-soluble species from which the correction factor is determined, adjustments to the factor must be made to correct for sodium binding to carbon dioxide (bicarbonate) and to protein. Referring also to Fig. 3, sodium concentration in the plasma is measured in the undiluted sample ($[Na]_d$ 40) by direct potentiometry with an ion selective electrode 60. Sodium concentration is measured using the diluted sample ($[Na]_i$) 42 by indirect potentiometry 62. Total carbon dioxide concentration [TCO_2] 44 is measured by a gas sensor 64. Protein concentration [TP] 46 is determined spectrophotometrically 66.

A plasma water correction factor is calculated, using equation 48, to be

$f = ([Na]_d/[Na]_i)\ (1 + [TCO_2]/\text{mole/l} + [TP]/600\text{g/dl})$.

The determined values are substituted into the above equation with the following modifications: The standard units for [TCO_2] are mmole/l, so the received concentration must be divided by 1000 before use. The standard units for [TP] are g/dl, so the received concentration can be used directly. If the analyzer does not measure the concentration of protein, the value [TP] can be set to 6 to reflect the average normal protein concentration.

Glucose concentration in the plasma sample is determined indirectly 50 using the diluted sample, by colorimetry or amperometry 68. The measured glucose concentration 50 is then multiplied by the calculated correction factor to get the corrected glucose concentration 52. Computations are performed by a computer 33 in analyzer 32 (Fig. 2).

To verify the accuracy of the correction factor, the concentration of glucose was measured directly using an enzyme electrode, in the presence of CO_2 (bicarbonate) and of several different concentrations of bovine serum albumin, and the measured values were compared with those obtained using the calculated correction factor to adjust the indirect measurement. The results are presented in the following table:

Sample No.	1	2	3	4	5	6
BSA (g/dl)	0	3	6	9	12	18
bicarbonate (mM)	20	20	20	20	20	20
sodium direct (mM)	137.7	136.1	135.5	135.2	134.8	133.7
sodium indirect (mM)	140.6	136.9	133.0	129.4	127.1	120.0
(f)	.999	1.02	1.05	1.08	1.10	1.17
glucose indirect (mg/dl)	203	195	190	184	178	170
glucose indirect x (f) (mg/dl)	203	199	200	199	196	199
glucose direct (mg/dl)	202	201	202	201	201	200

It can be seen that, within experimental error, the method gives the same value as does the direct measurement of glucose concentration.

Other embodiments are feasible. For example, the method of calculating a correction factor to adjust indirect measurements of water soluble species is applicable to any species for which a method of indirect measurement exists (e.g., additional examples include urea, cholesterol, or lactate). Any species for which both a direct and indirect method of determination exists can serve as a reference species for calculation of the correction factor (e.g., additional examples include potassium, chloride, or even glucose). For each reference species used, appropriate adjustments must be made to the factor to reflect any complexing of the species to another water soluble species.

Claims

1. A method for determining the concentration of a first water soluble species dissolved in a water based component of a biological fluid that also includes a second water soluble species and a volume occupying component characterized by:
measuring the concentration of said first water soluble species using an original sample of said fluid that has been diluted by an amount of additional aqueous solution to form a diluted sample,
measuring a direct concentration of said second water soluble species in the original sample undiluted by said additional aqueous solution,
measuring an indirect concentration of said second water soluble species using the diluted sample, and
adjusting said measurement of concentration of said first water soluble species by a combination of the direct and indirect concentration measurements of said second water soluble species.

2. The method of claim 1, further characterized in that adjusting said measurement of concentration of said first water soluble species comprises calculating a correction factor based on a combination of said direct and indirect concentration measurements of said second water soluble species.

3. The method of claim 2, further characterized in that the correction factor comprises calculating the ratio of said direct concentration to said indirect concentration of said second water soluble species.

4. The method of any of the preceding claims, further characterized in that said first water soluble species comprises glucose.

5. The method of any of the preceding claims, further characterized in that said biological fluid comprises blood plasma.

6. The method of any of the preceding claims, further characterized in that said second species comprises sodium ion.

7. The method of any of the preceding claims, wherein said biological fluid also includes at least one additional water soluble species that complexes with the second water soluble species, said method being further characterized by:
measuring the concentration of said additional species in said fluid, and
adjusting said measurement of concentration of said first water soluble species based on said measured concentration of said additional species.

8. The method of claim 7, further characterized in that said additional species comprises protein.

9. The method of claim 7, further characterized in that said additional species comprises carbon dioxide.

10. The method of claim 2,further characterized in that said first water soluble species is glucose dissolved in a water-based component of blood plasma that also includes sodium ion as a second water soluble species and further includes protein as a volume occupying component,

measuring a direct concentration of sodium ion in a sample of said plasma that has not been diluted by said aqueous solution, to obtain a value $[Na]_d$.

measuring an indirect concentration of sodium ion in a sample of said plasma, using a sample of said aqueous solution diluted plasma to obtain the value $[Na]_i$, and

measuring the concentration of total protein in a sample of said plasma to obtain a value [TP], and determining said correction factor (f) as

$f = ([Na]_d/[Na]_i)\ (1 + [TP]/600g/dl)$.

11. The method of claim 10 further characterized by measuring the concentration of total carbon dioxide in a sample of said plasma to obtain a value $[TCO_2]$, and determining said correction factor (f) as

$f = ([Na]_d/[Na_i])\ (1 + [TCO_2]/mole/l + [TP]/600g/dl)$.

12. Apparatus for correcting an initial measurement of concentration of a first water soluble species dissolved in a water-based component of a biological fluid that also includes a second water soluble species and a volume occupying component, said initial measurement having been taken on a diluted sample of said fluid, said apparatus comprising means for measuring the concentration of said first water soluble species in said diluted sample, characterized by comprising:

means for measuring a direct concentration of said second water soluble species in an undiluted sample of said fluid and an indirect concentration of said second species using a diluted sample of said fluid, and

a computational unit comprising means for receiving said concentration, and for adjusting said initial measurement of concentration of said first water soluble species by a combination of said direct and indirect concentrations of said second species to obtain a corrected measurement of concentration of said first water soluble species.

13. The apparatus of Claim 12, further characterized in that means for adjusting said initial measurement of concentration of said first water soluble species comprises means for generating a correction factor based on a combination of direct and indirect concentration measurements of said second water soluble species.

14. The apparatus of Claim 13, further characterized in that means for generating a correction factor comprises means for forming the ratio of said direct concentration to said indirect concentration.

15. The apparatus of any of Claims 12 to 14, further characterized in that said apparatus comprises means for measuring the concentration of glucose as said first water soluble species.

16. The apparatus of any of Claims 12 to 15, further characterized in that said apparatus comprises means for measuring a direct concentration of sodium ion as said second species.

17. The apparatus of any of Claims 12 to 16, wherein said biological fluid also includes an additional water soluble species that complexes with said second water soluble species, said apparatus being further characterized in additionally including means for measuring the concentration of said additional species in said fluid, and in that said computational unit includes means for adjusting said initial measurement of concentration of said first water soluble species based on said measured concentration of said additional species.

18. The apparatus of Claim 17, further characterized in that said apparatus comprises means for measuring the concentration of protein as said additional species.

19. The apparatus of Claim 17, further characterized in that said apparatus comprises means for measuring the concentration of carbon dioxide as said additional species.

20. The apparatus of Claim 13, for correcting an initial measurement of concentration of glucose dissolved in a water-based component of blood plasma that also includes protein as a volume occupying component, further characterized in that said apparatus comprises an analyzer comprising

means for measuring the concentration of glucose as said first species in said diluted sample,

means for measuring a direct concentration of sodium ion as said second species in a sample of said plasma that has not been diluted, to obtain a value $[Na]_d$,

means for measuring an indirect concentration of sodium ion in a sample of said plasma, using a sample of said plasma that has been diluted, to obtain a value $[Na]_i$, and

means for measuring the concentration of total protein in a sample of said plasma to obtain a value [TP], and

a computational unit comprising means for receiving said values and for determining a correction factor (f) as

$f = ([Na]_d/[Na]_i)\ (1 + [TP]/600g/dl$

and for adjusting said initial measurement of concentration of glucose by said factor.

21. The apparatus of Claim 20, further characterized in that said analyzer also includes means for measuring the concentration of total carbon dioxide in a sample of the plasma to obtain a value $[TCO_2]$, and said computational unit includes means for determining said correction factor (f) as

$f = ([Na]_d/[Na]_i)\ (1 + [TCO_2]/mole/l + [TP]/600g/dl)$.

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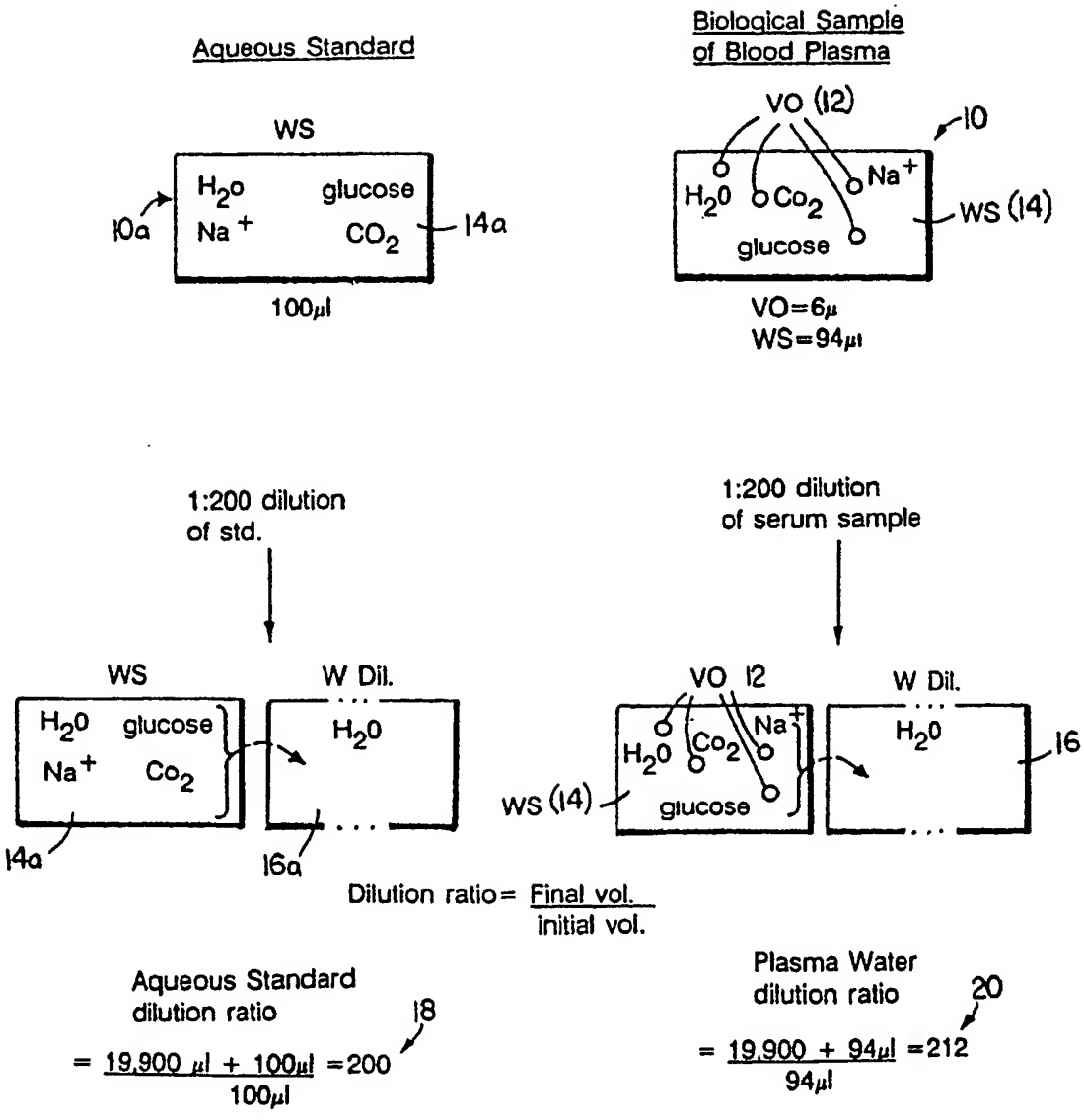

FIG. 1
(prior art)

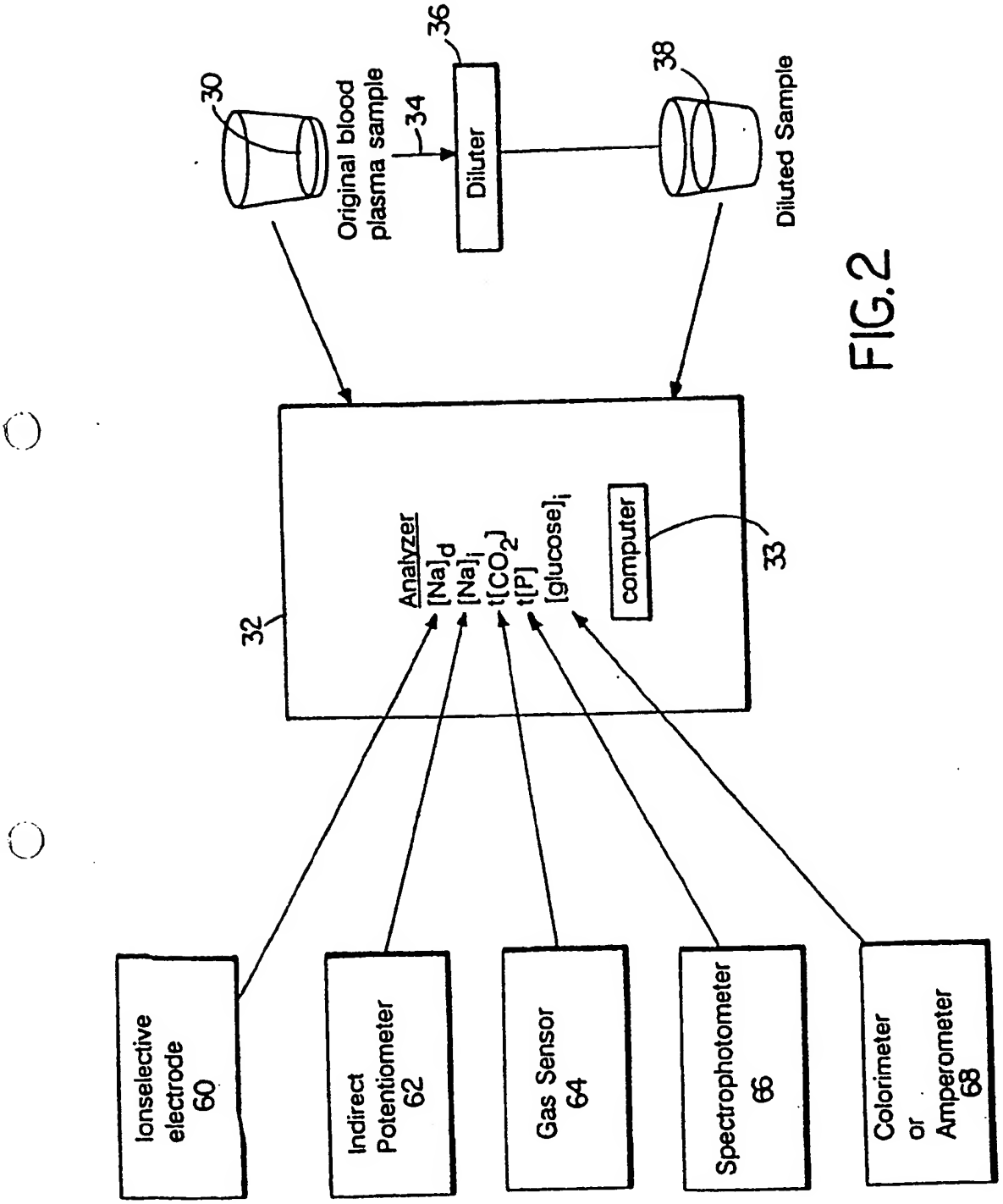

FIG.2

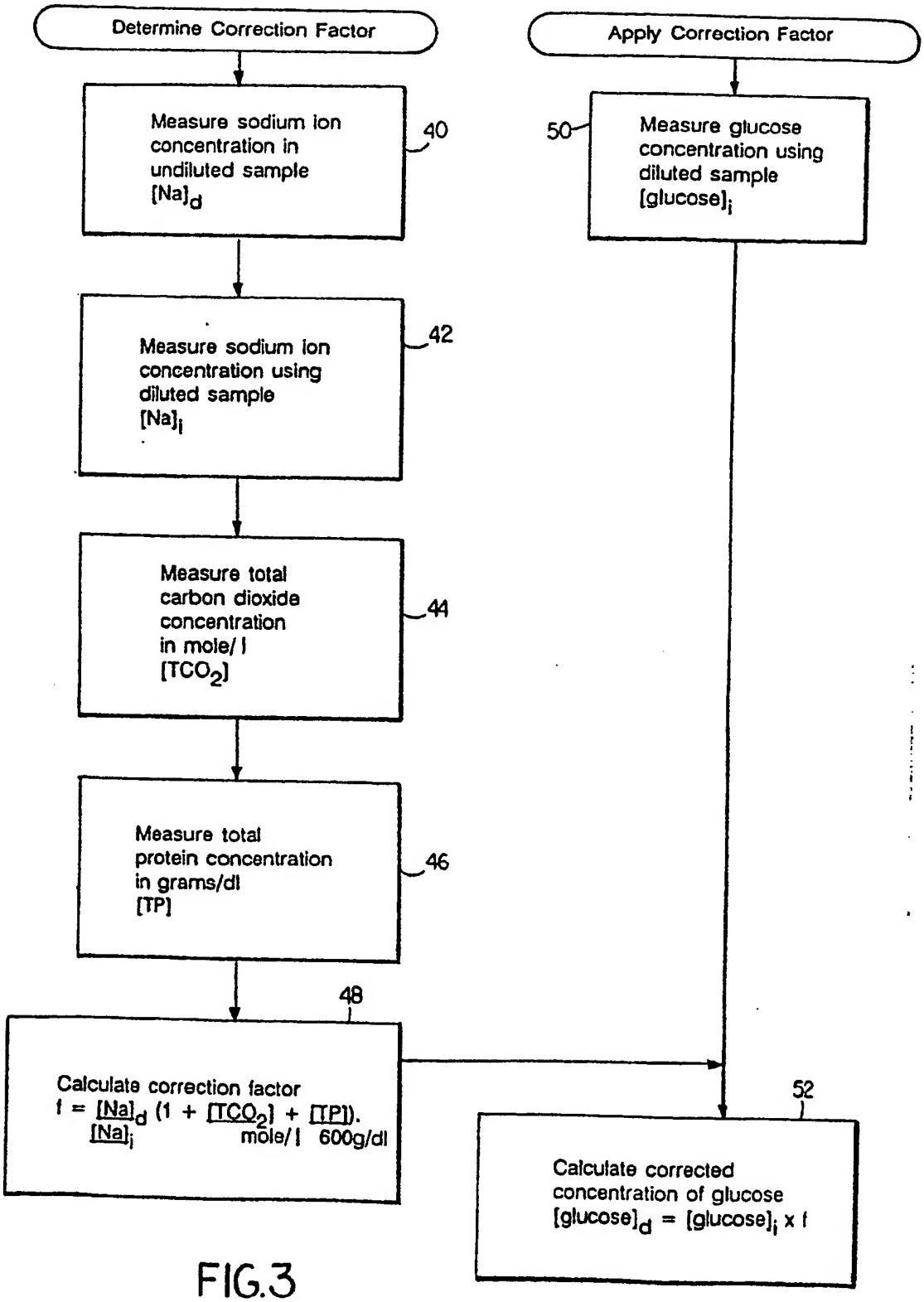
Determine Correction Factor
Measure sodium ion concentration in undiluted sample $[Na]_d$
40
Measure sodium ion concentration using diluted sample $[Na]_i$
42
Measure total carbon dioxide concentration in mole/l $[TCO_2]$
44
Measure total protein concentration in grams/dl [TP]
46
48
Calculate correction factor $f = \frac{[Na]_d}{[Na]_i} (1 + \frac{[TCO_2]}{\text{mole/l}} + \frac{[TP]}{600\text{g/dl}})$.
Apply Correction Factor
50
Measure glucose concentration using diluted sample $[glucose]_i$
52
Calculate corrected concentration of glucose $[glucose]_d = [glucose]_i \times f$

FIG.3

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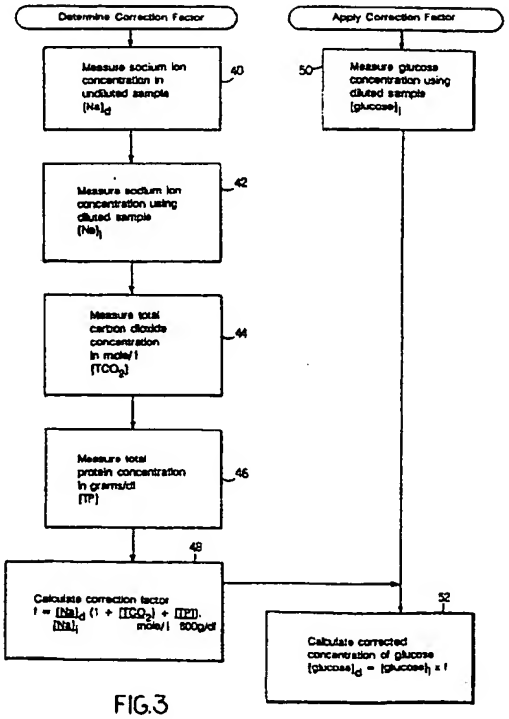

FIG.3

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EUROPEAN SEARCH REPORT

Application Number

EP 90 31 0175

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A,D	CLINICAL CHEMISTRY vol. 26, no. 10, 1980, page 1517; C.SHYR ET AL.: 'Effect of Dample Protein Concentration on Results of Analyses for Sodium and Potassium in Serum.' * the whole document *	1-10	G01N33/48
A,D	CLINICAL CHEMISTRY vol. 27, no. 11, 1981, pages 1938 - 1939; R.L.COLEMAN ET AL.: 'Evidence for Formation of Bicarbonate Complexes with Na+ and K+ under Physiological Conditions.' * the whole document *	1-10	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	29 APRIL 1992	HITCHEN C.E.

CATEGORY OF CITED DOCUMENTS

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